Studies on A Mosaic Inducing Virus on Radish (Raphanus sativus L.).

DISSERTATION SUBMITTED
TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF PHILOSOPHY

IN

BOTANY

BY

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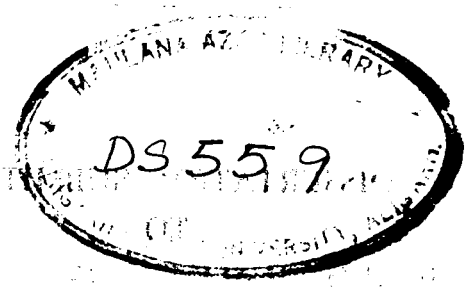
ALIGARH

October, 1983



CERTIFICATE

This is to certify that the dissertation entitled "Studies on a mosaic inducing virus on radish (*Raphanus sativus* L.)" submitted to the Aligarh Muslim University, Aligarh, in partial fulfilment of the requirements for the award of the degree of Master of Philosophy is a faithful record of the bonafide research work carried out by Mr. Jawaid A. Khan. No part of the dissertation has been published or submitted for any other degree or diploma.

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ACKNOWLEDGEMENTS

I wish to express my heart felt sense of gratitude to my Supervisor, Dr. Qamar A. Naqvi, Lecturer, Department of Botany, Aligarh Muslim University, Aligarh, under whose guidance and kind supervision the present work was carried out.

My sincerest thanks are due to Dr. Khalid Mahmood, Reader, Department of Botany, for his affection, constant help, advice and going through the manuscript, who has always been a source of inspiration to me.

I am also greatly indebted to Prof. S.K. Saxena, Chairman, Department of Botany, Aligarh Muslim University, Aligarh, for providing necessary research facilities.

I wish to express my appreciation and thanks to Messrs S.Husai F.N. Siddiqui, M.K.A. Jabri, I.H. Zaidi, K.A. Siddiqui, M. Aslam and T.A. Khan for their help during the preparation of this manuscript.

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CONTENTS

Chapter 1

INTRODUCTION

Radish is grown all over the world and is a favourite vegetable with all races. In India, it constitutes one of the most important vegetables, so much so that throughout the year it is the inalienable part of diet of Indian people.

From the commercial point of view, radish is a profitable crop. The vegetable has a short crop period. It is easy to raise, requires not much care and investment and is amply productive.

Radish is credited with refreshing and depurative properties. Radish preparations are useful in liver and gall bladder troubles. In homoeopathy they are used for neuralgic headaches, sleeplessness and chronic diarrhoea. The roots are said to be useful in urinary complaints, piles and in gastrodynia. The juice of fresh leaves is used as diuretic, laxative and lithotriptic.

Radish seeds contain glycosidically bound mustard oils of which sulphopropane shows antibacterial activities against Streptococcus, Pyococcus and Escherichia coli. The seeds also contain an antibiotic named raphrolysin, specific against Mycobacterium tuberculosis.

The leafy tops of radish are a good source of Vitamins and minerals. Protein is also extracted from radish on commercial scale.

Many diseases including fungal, bacterial, viral and physiological disorders cause a lot of reduction in the yield of radish. Viral diseases are quite important as they not only affect the quality of radish but also render the whole crop un-marketable.

Ahlawat and Chenulu (1982) reported that this disease causes losses ranging from 3.3 to 100 per cent in root weight and upto 95.8 per cent in seed weight depending on the time of infection.

A mosaic disease of radish was described by Kulkarni (1924) from Poona, India. Since then this disease has been reported from many parts of the world, including India. The symptoms of the disease include mosaic mottling of young leaves often associated with circular interveinal chlorotic areas which gradually increase in size and finally coalesce to form irregular patches. The affected plants are stunted and the leaves reduced in size. Frequently, lesions appear on the midrib, the leaf first bends outward and then the midrib breaks at the necrotic region so that a part of leaf collapses.

As is evident from the foregoing description, radish is used as food and medicine. Any abnormality or deficiency in the same would greatly affect its value as a vegetable food. From the commercial point of view the disease also reduces its market value. Thus, the mosaic disease of radish is a menace for the consumer as well as the farmer.

The virus causing the disease should, therefore, be throughly investigated. As such, properties of the incitant and its mode of transmission should be known so that effective measures against the spread of the disease could be taken.

Chapter 2

REVIEW OF LITERATURE

A mosaic disease of radish was described as early as 1924 by Kulkarni from Poona, India. During the early stage of infection the disease caused mottling on leaves, stem and pods. Later on, the symptoms consisted of blanching, blistering, distortion of leaves and the pods, checking of flower and fruit formation and stunting of the plants.

Ogilvie (1928) reported a mosaic disease on wild and garden radishes (Raphanus-raphanistrum and R. sativus, respectively) from Bermuda. The disease caused distortion of the leaves, often with the production of blister like areas.

Dana and McWhorter (1932) recorded a mosaic disease of horse radish from Washington. The disease was transmitted to turnip and mustard where it produced a mosaic similar to horse radish. The plants became dwarfed and many died within a short time. At digging time the plants were dwarfed and yellowed. Roots averaged small, had rough scaly surfaces and were unsalable because of pithy textures and frequent dark streaks. Root cuttings of diseased radishes were potted and forced at different temperatures. The young leaves exhibited a prominent mosaic like mottle, characterised by interveinal pale green areas, interspersed with dark green. Old leaves developed black elongated lesions in the epidermis and outer cortex of the petioles. The foliage was more or less stunted and leaf blades were strikingly segmented in a fern like manner.

In San Francisco, Bay Section of California, U.S.A., Tompkins (1939) found the occurrence of a mosaic disease of radish. Irregular shaped chlorotic lesions were developed on diseased leaves and later on they developed into a coarse mottle on older infected plants. The normal dark green tissues appeared as irregular shaped non-raised islands on a yellowish green chlorotic back ground. The virus was readily transmitted mechanically to Chinese-cabbage, sprouting broccoli, cauliflower, kohl-rabi, black and white mustard, chinese radish, lambs-quarters, sowbane, spinach, rocket larkspur N. glutinosa, N. langsdorfii, N. tabaccum var. Turkish and White Burley in addition to several cruciferous weeds. The virus remained infective for 14 days at 22°C and it lost its infectivity by heating at 68°C for 10 min. The virus retained infectivity to a dilution of 1 : 14000. Aphids (Brevicoryne brassicae, Lipaphis pseudobrassicae and Myzus persicae) failed to transmit this virus.

Severin and Tompkins (1950) studied the transmission of radish mosaic virus by aphids. The virus was transmitted in a non-persistant manner by Brevicoryne brassicae, Rhopalosiphum pseudobrassicae, Myzus ornatus, M. persicae, Aphis anii, A. gossypii A. rumicis, A. ferruginea-striata, Cavariella aegopodii, Macrosiphum pisi, M. solani (Nasonovia) and Myzus circumflexus. Percentage of transmission with the aphids given pre-acquisition starving was higher than with unstarved aphids. The aphids remained infective for three hours after the feeding commenced. Kasai (1950) reported the transmission of mosaic disease of Japanese radish by single aphid (M. persicae) feeding for 5 minutes on diseased plant and for the same period on healthy plants. Pre-

acquisition starving of one hour greatly enhanced the efficiency of the vector. The aphids retained infectivity for three hours but not for five hours.

Takahashi (1952) reported the occurrence of rod shaped particles 105-120 x 25 mm in extracts of white icicle radish, tendergreen mustard (Brassica juncea) and shogoin turnip, infected by radish mosaic virus.

Raychaudhuri and Pathanian (1955) described a mosaic disease of radish, which was first observed at the I.A.R.I., New Delhi, in 1951. The disease was accompanied by necrosis and stunting. The virus infected the plants of family Cruciferae only. The symptoms consisted of mosaic mottling on young leaves often associated with circular interveinal chlorotic areas which gradually increased in size and finally coalesced to form irregular chlorotic patches. The affected plants had reduced leaves and were stunted. The virus survived at 85°C but not at 90°C, at a dilution of 1 : 10,000, and for 17 days at 17-22°C and 101 days at 6-8°C. Seed transmission was absent.

Yamaguchi (1960) studied the viruses producing mosaic in radish, in Central Region of Japan. Cucumber mosaic virus (CMV) was identified in 13 of the 20 collections while radish mosaic virus was isolated from one collection which also contained CMV.

A comparative study of several radish viruses in parallel experiments with known turnip mosaic virus and cauliflower mosaic virus group was done in an attempt by Horton et al. (1961) to elucidate the relationship of radish mosaic virus to these groups.

Kou (1961) described a mosaic disease of radish from Taiwan. The symptoms included a systemic vein clearing and general mottling. The virus was inoculated mechanically to radish, chinese cabbage, chinese mustard, swede, turnip, white-Burley and Turkish tobacco, Nicotiana glutinosa and N. rustica. In greenhouse it was also transmitted by Brevicoryne brassicae, Myzus persicae and Rhopalosiphum pseudobrassicae, but not by seeds. Longevity was between 48 and 72 hrs. at 20-22° C, thermal inactivation point was at 55° C for 10 minutes and dilution end point 1 : 3000-4000.

Joshi (1962) studied a mosaic disease of radish which produced symptomless, local infection on N. glutinosa and N. rustica. Infected seed beds of stock (Mathiola incana) candy tuft (Iberis sp and sweet rocket (Sisymbrium sp.) were the reservoir hosts. The virus was transmitted by Aphis gossypii and M. persicae in a non-persistant manner but not by seeds and was identified as a strain of cabbage black ring spot virus.

Joshi and Bhargava (1963) recorded the natural occurrence of cabbage black ring spot virus on Brassica juncea and Lepidium ruderale (a perennial weed in parts of Kumaon district) besides radish. Removal and destruction of this weed (L. ruderale) and self sown seedlings of cultivated crucifers (which harbour the virus during unfavourable periods) were recommended as control measures. Campbell (1964) isolated a virus from turnip in California and proposed the name radish mosaic virus (RaMV) because of the similarities to the radish mosaic virus of Tompkins (1939) in host range, host reaction, physical properties and apparent

lack of aphid transmission. This RaMV was serologically related to bean pod mottle virus, Arkansas, cowpea mosaic virus and squash mosaic virus and it had polyhedral particles, 30 nm in diameter. No serological reaction was detected when RaMV was tested against antisera to 11 other viruses including turnip yellow mosaic virus and turnip crinkle virus.

Li and Cheo (1964) recorded mixed infection on radish. The viruses identified were two strains of turnip mosaic virus, two strains of cucumber mosaic virus and a virus causing ring spot on Nicotiana glutinosa. Of 68 isolates, 20 from 10 field specimens were found to be mixed. The two strains of cucumber mosaic virus and the ring spot virus were not transmissible by sap or Myzus persicae to Peking cabbage, radish or chinese rape while cucumber mosaic virus strains and the ring spot virus were infective to radish only when inoculated simultaneously.

Campbell and Colt (1967) studied the transmission of RaMV and found it to be inefficiently transmitted by Phyllotreta sp. and Diabrotica indecimpunctata.

Iochihara (1968) reported enation mosaic virus from Japan on radish. Symptoms developed were enation mosaic, necrosis and distortion of the leaves. While turnip developed necrotic local lesions and systemic mosaic, necrotic local lesions were produced on Chenopodium amaranticolor. Petunia was a symptomless carrier. The virus showed some similarities with radish mosaic virus but differed in its symptoms on radish and lack of infectivity to tobacco.

Stefanac et al. (1971) reported the occurrence of a strain of radish mosaic virus on turnip from Yugoslavia. Yugoslavian isolates resembled the type strain in host range, symptoms, physical properties, particle morphology and transmission by Phyllotreta undulata. It was serologically related to the type strain but did not infect radish and was designated as European strain. Characteristic inclusion bodies were seen in the cytoplasm of infected turnip and some cruciferous spp.

Honda and Matsui (1972) saw the masses of radish mosaic virus particles on the interface between cytoplasm and vacuole or in the vacuole under electron microscope. Within tonoplast the virus particles were aligned on multirows. The virus particles were also seen in plasmodesmata across the cell wall.

Hooper et al. (1972) reported the development of enation at the flowering time on the under-surface of leaves in Chinese white winter radish plants infected with RaMV. Virus like particles 24-26 nm in diameter were present in chlorotic tissues or near necrotic spots in enation or other areas of leaves.

Kamula et al. (1972) reported the radish mosaic virus for the first time on turnip from Hungary. Swede, cauliflower, Brussels sprouts and Sinapsis arvensis were recorded as natural hosts in Yugoslavia. The isolates from the two countries were serologically similar.

Shukla and Schmelzer (1973) studied the occurrence of turnip yellow mosaic virus and radish mosaic virus in German Democratic Republic. Thirteen ornamentals and wild crucifers were found as

spontaneous hosts of turnip yellow mosaic virus and three Nicotiana sp. for radish mosaic virus. The two isolates differed from foreign strains and also from one another.

Horvath et al. (1973) described Crambe abyssinica as a differential host for the screening of turnip mosaic virus and radish mosaic virus. Y65 and B4 strain of turnip yellow mosaic virus induced bright yellow mosaic on uninoculated leaves and axillary shoots. This intensified and grey brown irregular islands developed on this plant while HZ and M7 strains of radish mosaic induced fine vein clearing and vein necrosis on non-inoculated leaves and reduced inflorescence, leaf size and plant growth.

Jurectic and Fulton (1974) purified the HZ strain of radish mosaic virus using 0.03M $Na_2B_4O_7$ at pH 6. The virus was separated into 4, 3, or 2 zones in sucrose density gradient centrifugation. Of 4 particles types (Ta, T, M and B from top to bottom) only B was infectious. The size of B particles was 28 nm and of others it was 23 nm.

Shukla and Schmelzer (1974) purified the GDR strain of radish mosaic virus and compared it with an American and a Yugoslavian isolate. The three isolates were found to be distinct strains

The serological and other relationships among the isolates of radish mosaic virus were studied by Plakolli and Stefanac (1976) The isolates belonged to either the neotype strain reported for the first time from California (ATCC 441809) or the European strain described from Yugoslavia (RPP 51,2954). The Japanese isolate from

radish (RAM 47,2309) may be added to the neotype strain and European isolates B(RPP 53,309), Kv (RPP 53,453), DE_4 (RPP 53,404) and S (RPP 54,262) to the typical European strain.

A mosaic disease of garden radish was described by Schmelzer (1976) from E. Germany. Cauliflower mosaic virus was isolated from diseased plants.

Joshi (1977) studied the natural occurrence of turnip mosaic virus on radish in Kumaon district from India. He showed that Anhis gossypii played a very important role in the epidemiology of this virus. Myzus persicae, Linaphis erysinii, Anhis gossypii and A. craccivora transmitted the virus in the radish fields.

Sakai and Kono (1978) reported the occurrence of turnip mosaic virus in weeds around radish fields serving as important source of inoculum for radish crops. Among the naturally infected weeds the virus was isolated from Cerastium glomeratum and in this weed TuMV was found to overwinter.

Horvath (1979) separated 22 viruses with the use of differential hosts. He showed that Linantia erecta was susceptible to TuMV and resistant to both radish mosaic virus and turnip yellow mosaic virus and Nicotiana occidentalis, Tetragonia crystallnia and T. echinata were susceptible to RaMV and turnip mosaic virus and were important in the identification of the three viruses.

Natsuaki et al. (1980) described radish yellow edge virus (RYEV) in young seedlings of Japanese radish. RYEV was transmitted through seeds. RYEV infected seedlings showed mild symptoms of

yellow edge and dwarfing in lower leaves. The purified preparation showed an UV absorption spectrum characteristic of a nucleoprotein. The sedimentation constant was 113S. Inclusion bodies containing RYEV particles were observed in the cytoplasm and phloem parenchyma cells. RYEV particles were 30 nm in diameter. RYEV was detected in apparently healthy seedlings from seeds of carrot, spinach, leaf beet (Beta vulgaris var. cicla) and table beet (B. vulgaris var. esculenta cv. Detroit Dark Red).

Ahlawat and Chenulu (1982) studied the virus vector relationship between radish mosaic virus and the aphid Lipaphis erysimii (Kalt). They found maximum efficiency after one hour starvation. The optimum acquisition and transmission access periods were two minutes and one hour respectively. There was increase in transmissic with the increase in number of viruliferous aphids per plant. The virus was of non circulative/non persistant type.

Chapter 3

MATERIALS AND METHODS

Cultivation of plants

All plants were grown in pots in a mixture of sand, soil and compost. Soil was sterilised by autoclaving for 1 hr at 20 lb pressure. Soil was kept over night at room temperature after autoclaving. Clay pots of 4 inches and 6 inches diameter were sterilised with 5 per cent formaldehyde solution. Nicotiana glutinosa plants to be used as a virus source and Chenopodium amaranticolor plants as test plants, were raised in wooden trays in autoclaved soil. When plants were at 3 leaf stage (about 2 weeks old), they were transplanted singly in clay pots. Plants were used for inoculation 2 weeks after transplantation. All plants were raised and kept in an insect proof glasshouse at a temperature of 20-30° C.

Virus culture and method of inoculation

The isolate of mosaic inducing virus on radish used in these studies was obtained from a diseased radish plant, Raphanus sativus L. showing mosaic symptoms and stunted growth of plants in Kasimpur, Aligarh. Culture was maintained by manual sap inoculation to Nicotiana glutinosa plants. Sap from the diseased plants was extracted by macerating young leaves of Nicotiana glutinosa inoculated two weeks earlier in a mortar with pestle after addition of 0.1M phosphate buffer pH 7.0. For each gram of plant material 2 ml of buffer was used. Sap was obtained by squeezing the macerate

through two layers of cheese cloth. This extract was routinely used as inoculum for maintaining the virus culture. The three lowermost leaves were inoculated with the extract using the forefinger and employing carborundum 800 mesh as an abrasive. Leaves were rinsed with a stream of water immediately after inoculation.

Host range studies

Several species of plants were inoculated with the isolate using sap from N. glutinosa inoculated 10-12 days earlier. Plants were raised in clay pots 4" and 6" in diameter. Inoculations were done manually using the forefinger and carborundum 800 mesh as an abrasive. Plants at 3-4 leaf stages were used for inoculation. All the fully expanded leaves were inoculated. The inoculated leaves were rinsed with a stream of water soon after inoculation. At least 3 plants of each species were inoculated and the same number of plants were kept as control. After inoculation plants were observed daily for the development of symptoms. Time, sequence and severity of symptoms were noted. The plants were kept under observation for 8 weeks. Back inoculation was made on Chenopodium amaranticolor from all the plants.

Concentration of the virus in the plant

For studying the changes in the concentration of the virus in N. glutinosa plant at different intervals after inoculation, seedlings were raised in wooden trays. Seedlings of uniform growth were selected 2 weeks after sowing and transplanted singly in 4"

clay pots. Two weeks after transplantation 25 plants of uniform growth and size were used for inoculation. Young leaves, from the apical portion of one plant were harvested after 2 days and macerated in a mortar with pestle after addition of 0.1M phosphate buffer at pH 7.0 (1 gm : 2 ml). Sap was expressed by squeezing the macerate through cheese cloth and inoculated to six C. amaranticolor plants. This process was repeated at intervals of 2 days upto 26 days.

Thermal inactivation

The experiments were done on C. amaranticolor plants. Sap was obtained by crushing young leaves of N. glutinosa plants inoculated 10-12 days earlier in a mortar with pestle. Sap was obtained by filtering through cheese cloth. It was divided in aliquots of 5 ml and put in glass vials. The vials were held in a water bath in such a way that the sap level was slightly below the water level in the bath and heated upto the required temperature. The sap in the vials was heated for 10 min at 40, 45, 50, 55, 60, 65 and 70°C. The vials were cooled by dipping in cold water immediately after removal from the water bath. The aliquots were then inoculated to plants manually employing carborundum 800 mesh as an abrasive. The inoculated leaves were rinsed with a stream of water after inoculation. Six plants were inoculated with each aliquot. The experiment was repeated thrice.

Dilution end point

Young leaves of diseased plants inoculated 10-12 days earlier

were macerated in a mortar with pestle and the sap filtered through two layers of cheese cloth. Ten fold dilutions of the sap were made in double distilled water. Leaves of C. amaranticolor plants were inoculated with different dilutions of sap using carborundum 800 mesh as an abrasive. The inoculated leaves were rinsed immediately after inoculation with a gentle stream of water. Six plants were inoculated with each dilution. The experiment was repeated thrice.

Longevity in vitro

Infected leaves of plants inoculated 10-12 days earlier were macerated in a mortar with a pestle. Sap was filtered through cheese cloth. Aliquots from the sample stored at room temperature i.e. (25 ± 5°C) were inoculated at intervals of 6 hr to C. amaranticolor plants. The experiment was repeated thrice.

Rearing of the aphids

Colony of virus free aphids Myzus persicae Sulz. and Macrosiphoniella sanbornii Gill. was started from newly born nymphs. Viviparous adults were starved for 2-4 hr and thereupon placed on a healthy detached leave of Nicotiana tabacum in a petridish. During the next 1-2 hr nymphs were born at a rate of 2-3 per adult. The newly born nymphs were transferred to a fresh healthy plant at an interval of a few minutes. The colonies were maintained at a temperature of 20-22°C under long day conditions. A continuous colony of these aphids was maintained by removing three old plants and adding three fresh plants each week.

These virus free aphids (Myzus persicae Sulz.,) were reared on Brassica oleraceae and Macrosiphoniella sanbornii Gill. were reared on Chrysanthemum spp.

Aphid transmission

Nymphs were collected from the healthy colony with a camel's hair brush. About 100 aphids collected from the healthy colony were starved for 1 hr and then colonized on N. glutinosa plants inoculated 10-12 days earlier. N. glutinosa plants were covered with Lentz plastic cages. In one set of experiment 5 batches of 10 aphids each were removed from the plants after allowing acquisition feeding time of 2, 4, 6, 8 and 10 minutes respectively and were then placed on very young N. glutinosa plants. They were then allowed the same inoculation feeding time as the acquisition feeding time. The aphids were killed by 0.2 per cent Dimecron spray. The plants were transferred on the glasshouse bench and symptoms were observed periodically and recorded.

Effect of pH on infectivity

Young leaves from plants inoculated 10-12 days earlier were harvested and macerated in a mortar with pestle. Sap was filtered through two layers of cheese cloth and pH of the sap adjusted to 5.0 and 6.0 with 0.1M acetic acid and 7.0, 8.0 and 9.0 with 0.1M NaOH. Sap was inoculated to the leaves of six C. amaranticolor plants. The experiment was repeated thrice.

Effect of various buffers at different pH levels on infectivity

In this experiment the effect of different buffers at various pH levels on the infectivity of the virus was investigated. Lots of infected leaves from apical portion of the plant inoculated 10-12 days earlier were homogenised in a mortar with pestle after addition of 0.1M acetate, phosphate and borate buffer at pH values of 5.5, 6.0, 7.0, 7.5, 8.0 and 8.5. The homogenate was filtered through two layers of cheese cloth and then inoculated on to the leaves of C. amaranticolor plants using six plants for each pH level. Inoculation was done manually using carborundum 800 mesh as an abrasive. The plants were washed with a gentle stream of water after inoculation. The experiment was repeated thrice.

Chapter 4

RESULTS

Host range studies

An experimental host range of mosaic inducing virus on radish was studied. Manual sap inoculations were done at different times of the year to determine seasonal variations. Back inoculations were made from all the plants including those which did not show any visible symptoms to check if any of them was a symptomless carrier of the virus. Ninety two plant species belonging to 22 familes were inoculated. Results are summarized in Table 1.

Table 1: Host range and symptoms of Radish mosaic virus on different hosts.

Species	Symptoms	Back inoculation
Acanthaceae		
Peristrophe bicalyculata Nees.	-	-
Amaranthus caudatus L.	-	-
Amaranthus gracilis Desf.	-	-
Amaranthus tricolor L.	-	-
Gomphrena globosa L.	-	-
Aizoaceae		
Tetragonia expansa Murr.	LL	-

Species	Symptoms	Back inoculation
Apocynaceae		
Catharanthus roseus G. Don.	-	-
Balsaminaceae		
Impatiens balsamina L.	-	-
Caryophyllaceae		
Dianthus barbatus L.	-	-
Dianthus caryophyllus L.	-	-
Chenopodiaceae		
Chenopodium album L.	-	-
C. ambrosoides L.	-	-
C. murale L.	LL	-
C. amaranticolor Coste & Reyn.	LL	-
Beta vulgaris saccharifera L.	NLL	-
Spinacia oleracea L.	-	-
Commelinaceae		
Commelina nudiflora L.	-	-
Compositae		
Ageratum conyzoides L.	-	-
Aster indicus L.	-	-

Species	Symptoms	Back inoculation
Calendula officinalis L.	-	-
Cineraria chinensis Sprng.	-	-
Cosmos bipinnatus Cav.	-	-
Dahlia pinnata Cav.	-	-
Launaea asplenifolia Hook.	-	-
Sonchus asper Hill.	-	-
Tagetes erecta L.	-	-
Chrysanthemum segetum L.	-	-
Helianthus annuus L.	VC, MOS	+
Cruciferae		
Brassica campestris		
var. Type - 59	VC, MOS, LD	+
var. PR - 15	VC, MOS, LD	+
var. Pusa Bold	VC, MOS	+
Brassica oleracea L.		
var. botrytis L. cv. Late Patna	-	-
var. Giant Snowball	-	-
B. pekinensis var. Wong Bok	-	-
Brassica oleracea L.		
var. Capitata Knol khol.	-	-
var. King of the Market	-	-
var. Early White Vienna	-	-
Brassica rapa L.	-	-
Raphanus sativus L.	VC, MOS, MOA, LD	+

Species	Symptoms	Back inoculation
Cucurbitaceae		
Benincasa hispida Cogn.	VC, MS	+
Citrullus vulgaris Schard.	-	-
Coccinia cordifolia Cogn.	-	-
Coccinia indica Wight and Arn.	-	-
Cucumis melo L.	-	-
Cucurbita moschata Duchens.	-	-
Luffa cylindrica Roem.	-	-
Lagenaria leucantha (Duch.) Rusby.	-	-
Graminae		
Pennisetum typhoides Burm.	-	-
Hordeum vulgare L.	-	-
Zea mays L.	-	+
Labiatae		
Coleus blumei Benth.	-	-
Leguminosae		
Cajanus indicus Spreng	-	-
Crotalaria medicaginea Lam.	-	-
Dolichos lablab (Roxb.) & L.	-	-
Phaseolus mungo L.	-	-
Phaseolus radiatus L.	-	-

Species	Symptoms	Back inoculation
Pisum sativum Linn.	-	-
Trigonella foenum graecum L.	LL	-
Vicia faba L.	LL	-
Vigna sinensis Endl.		
var. P_{3-1}	-	-
var. Pusa Bursati	-	-
Malvaceae		
Abelmoschus esculentus (L) Moench.	-	-
Nyctaginaceae		
Mirabilis jalapa L.	-	-
Polemoniaceae		
Phlox drummondii Hook.	-	-
Ranunculaceae		
Delphinium ajacis L.	-	-
Scrophulariaceae		
Antirrhinum majus L.	-	-
Solanaceae		
Capsicum annuum L.		
var. Bull Nose	VC, MOS, LD,S	-

Species	Symptoms	Back inoculation
var. California Wonder	VC, MOS, LD, S	-
var. Elephant Trunk	VC, MMOS, DC, S	-
var. G-4	VY, MMOS, DC, S	-
var. Hot Portugal	VY, MOS, LD, S	-
var. Hungarian Wax	VC, MMOS, S	-
var. Jwala	VC, MMOS, LD, S	-
var. New Red Hot	VC, MOS, LD, S	-
var. Surya Mukhi	VY, MOS, S	-
var. Surya Mukhi Black	VY, MMOS, S, LD	-
var. Surya Mukhi Green	VC, MOS, S	-
Datura stramonium	-	-
Lycopersicon esculentum Mill.		
var. Pusa Ruby	-	+
var. Marglobe	VC, MOS	+
Nicotiana glutinosa L.	VC, MMOS, LD, SSS, E	+
Nicotiana tabacum L.		
var. Harrisons Special	VC, MMOS, LD	+
Var. T. Samsun	MMOS, S	+
var. T. Xanthi	VY, MMOS, LD, S	+
var. Anand-2	VY, MOS, S	+
var. White Burley	VC, MOS	+
var. Bhopali	VY, MOS, YP	+
var. GI_4	VC, MMOS, S	+
var. NP-37	VC, MOS, S	+

Species	Symptoms	Back inoculation
P. plumbaginifolia Willd.	VC, MOS, S	+
Nicotiana rustica L.	VC, MOS, LD	+
Solanum melongena L.		
var. Black Beauty	VC, MOS, DC, S	+
var. Long Black	VY, MOS, S	+
var. Long Green	VY, MOS, S, DC	+
var. Pusa Purple Long	VY, MOS, S	+
var. Pusa Kranti	VY, MOS	+
Solanum tuberosum L.	CLL	+
Petunia hybrida Vilm.	-	+
Solanum nigrum L.	-	-
Trapaeolaceae		
Trapaeolum majus L.	LL	-
Umbelliferae		
Anium graveolens L.	-	-
Ammi majus L.	-	-
Daucus carota L.	-	-
Coriandrum sativum L.	-	-
Verbenaceae		
Verbena hybrida Cogn.	MOS, LD, S	+

+ = Recovery of the virus on back inoculation to local lesion host.
- = No symptoms and no recovery of the virus on back inoculations to local lesion host.

CLL = Chlorotic local lesion.
DC = Downward curling.

E = Enation.

LD = Leaf deformation.

MMO = Mosaic mottling.

MO = Mosaic.

NLL = Necrotic local lesion.

LL = Local lesions.

RGA = Raised Green areas

S = Stunting.

SSF = Shoe string formation.

VC = Vein clearing.

VY = Vein yellowing.

Concentration of virus in N. glutinosa at different time after inoculation

The time at which infected tissue is harvested should be so chosen as to maximise the starting concentration of infectious virus. For many viruses concentration rises to a peak after a certain period and then falls rapidly. To determine the time after mechanical inoculation of N. glutinosa plants when the virus reached the highest concentration, tissue from N. glutinosa at intervals of 2 days after mechanical inoculation, was harvested and assayed. Table 2 shows that RaMV attained the highest concentration, 12 days after inoculation. The concentration fell slowly thereafter and after 20 days reached a very low level.

Table 2. Concentration of RaMV in Nicotiana glutinosa at different time after mechanical inoculation.

Days	No. of local lesion/leaf*
2	00
4	06
6	09
8	12
10	12
12	15
16	10
18	08
20	05
22	04
24	04
26	04

*Average of 6 plants having 6 leaves in each plant.

Properties of the virus in plant sap

It is desirable to study the physical properties of a virus in plant sap before an attempt is made to purify it and study its physicochemical properties. Information regarding the stability of virus, its dilution end point and thermal death point are of immense help in determining the procedure to be employed for its purification and further characterization.

Thermal inactivation point

In crude sap RaMV was still active after being heated for 10 min at 60°C but was inactivated at 65°C (Table 3).

Table 3. Thermal inactivation point of radish mosaic virus.

Temperature °C	Number of lesions/leaf*
Without treatment	20
40	20
45	15
50	04
55	03
60	03
65	00
70	00

*Average of 6 plants having 6 leaves each plant.

Dilution end point

The virus in crude sap was found infective upto a dilution of 10^{-3} but no local lesions were formed at a dilution of 10^{-4}. The dilution end point of RaMV is 10^{-4} (Table 4).

Table 4. Dilution end point of radish mosaic virus.

Dilutions	No. of lesions/leaf*
Undiluted	30
10^{-1}	15
10^{-2}	05
10^{-3}	02
10^{-4}	00
10^{-5}	00

*Average of 6 plants having 6 leaves in each plant.

Longevity in vitro

Retention of infectivity during storage of infective sap varies greatly with different viruses and plays an important role in studying its properties. Infectivity of RaMV was greatly affected during storage. The RaMV crude sap was stored at room temperature (25 ± 5°C) and was found to have a longevity in vitro for 36 hr but lost infectivity after a storage of 42 hr (Table 5).

Table 5. Longevity *in vitro* of radish mosaic virus.

Time in hrs.	No. of lesions/leaf*
6	18
12	08
18	02
24	02
30	01
36	01
42	00
48	00

*Average of 6 plants having 6 leaves in each plant.

Aphid trahsmission

Each plant was exposed to 10 aphids. All the aphids were given an initial fasting of 1 hr before the experiment. They were allowed the same inoculation feeding time as the acquisition feeding time. *Myzus persicae* and *Rhopalosiphum nymphaea* transmitted the virus while *Macrosiphoniella sanbornii* failed to transmit the radish mosaic virus. Results are given in Tables 6, 7 and 8.

Table 6. Transmission of radish mosaic virus by batches of 10 Myzus persicae Sulz., after a preliminary fasting of 1 hr.

Acquisition time (Min.)	Inoculation feeding time (Min.)	Infectivity*
2	2	3/10
4	4	8/10
6	6	6/10
8	8	5/10
10	10	3/10

* Numerator = Number of N. glutinosa plants infected.
Denominator = Number of N. glutinosa plants inoculated.

Table 7. Transmission of radish mosaic virus by batches of 10 Rhopalosiphum nymphaeae after a preliminary fasting of 1 hr.

Acquisition feeding time (Min.)	Inoculation feeding time (Min.)	Infectivity*
2	2	4/10
4	4	5/10
6	6	2/10
8	8	3/10
10	10	0/10

* Numerator = No. of N. glutinosa plants infected.
Denominator = No. of N. glutinosa plants inoculated.

Table 8. Transmission of radish mosaic virus by batches of 10 Macrosiphoniella sanbornii Gill. after a preliminary fasting of 1 hr.

Acquisition feeding time (Min.)	Inoculation feeding time (Min.)	Infectivity*
2	2	0/10
4	4	0/10
6	6	0/10
8	8	0/10
10	10	0/10

*Numerator = No. of N. glutinosa plants infected.
Denominator= No. of N. glutinosa plants inoculated.

Effect of pH on infectivity

Efforts were made to determine the most favourable pH for maintaining infectivity of the virus. Table 9 shows the results of infectivity measurements after the adjustment of the pH of the extracts. It is evident that pH 7.0 is the most suitable pH for maintaining the infectivity of the virus (Table 9).

Table 9. Effect of pH on the infectivity of radish mosaic virus.

pH	No. of lesions/ leaf*
5.0	11
6.0	23
7.0	38
8.0	21
9.0	09

* No. of 6 plants having 6 leaves in each plant.

Effect of various buffers at different pH levels on infectivity

In another experiment, the effect of different buffers at various pH levels, on the infectivity of the virus was compared. Results are summarised in Table 10. 0.1M phosphate pH 7.0 was found to be the most suitable medium for homogenising the infected tissue.

Table 10. The effect of different buffers at various pH levels on the infectivity of the radish mosaic virus.

Buffers	pH	No. of lesions/ leaf*
0.1M acetate	5.5	8
"	6.0	11
0.1M phosphate	7.0	48
"	7.5	34
0.1M borate	8.0	5
"	8.5	3

* Average of 6 plants having 6 leaves in each plant.

Chapter 5

DISCUSSION

A mosaic disease of radish (*Raphanus sativus* L.) characterized by mosaic mottling and raised green areas in the field-grown radish recurred year after year in Kasimpur, Aligarh. The virus causing disease was characterized using older criteria like host range, symptomatology and biophysical properties in crude sap.

The Aligarh isolate of radish mosaic virus infected 19 species of plants distributed in 10 families viz., Aizoaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Gramineae, Leguminosae, Solanaceae, Trapaeolaceae and Verbenaceae. It did not infect 54 other species distributed in 22 families. *Chenopodium amaranticolor*, *C. murale*, *Tetragonia expansa*, *Tropaeolum majus*, *Trigonella foenum-graecum* and *Vicia faba* were found to be good local lesion hosts for the isolate. *Lycopersicon esculentum* var. Pusa Ruby, *Petunia hybrida* and *Zea mays* were symptomless carriers. *Petunia hybrida* has already been reported as symptomless carrier by Tochihara (1968). However, *L. esculentum* var. Pusa Ruby and *Zea mays* are not reported as symptomless carriers by early authors.

The Aligarh isolate of RaMV is inactivated by heating at 65°C, rendered avirulent when diluted to 10^{-4} and when stored at room temperature for 42 hr. It attains highest concentration in *N. glutinosa* plants 12 days after manual sap inoculation. The most suitable pH for maintaining the infectivity was found to be

7.0 and out of several buffers tried at different pH levels phosphate buffer pH 7.0 provided the most suitable environment for maintaining the infectivity of RaMV. 0.01M was the most suitable ionic strength to maintain RaMV infectivity. Three species of aphids were tested in transmission studies of the virus. Myzus persicae and Rhopalosiphum nymphaea were able to transmit the virus in a non-persistant manner whereas Macrosiphoniella sanbornii failed to transmit the virus.

A comparison with other isolates of RaMV is made difficult due to lack of information regarding several properties of the isolates. The Aligarh isolate of RaMV resembles the virus described by Tompkins (1939) in several respects. Both the viruses infect several common hosts in Solanaceae and Cruciferae. Dilution end point and thermal inactivation point of both the viruses are about the same but they differ markedly by as far as ageing in vitro is concerned. Furthermore, Aligarh isolate is aphid transmitted whereas the virus described by Tompkins could not be transmitted by aphids.

The mosaic disease of radish described by Raychaudhuri and Pathanian (1955) is different from the one found in Aligarh. The former is confined to family Cruciferae and differs markedly in its physical properties in plant sap.

Radish enation mosaic virus (Tochihara, 1968) calls for no comparison as it is beetle transmitted whereas the Aligarh isolate is aphid transmitted.

RaMV resembles the virus described by Kou (1961) in having some common hosts, dilution end point and ageing *in vitro* but differs markedly by in thermal inactivation point.

The virus described from radish by Joshi (1962) was identified as a strain of cabbage black ring spot virus. It produced symptomless local infection on *N. glutinosa* and *N. rustica* which were found to be good systemic hosts for the Aligarh isolate.

Ahlawat and Chenulu (1982) studied virus vector relationship between radish mosaic virus and the aphid, *Lipaphis erysimii* and found the mode of transmission to be non-persistant type. The virus was also transmitted by *Myzus persicae*. The Aligarh isolate of radish mosaic virus is also transmitted by *Myzus persicae* and the mode of transmission was found to be non-persistant type.

No conclusion can be drawn as to the identity of the virus causing mosaic disease of radish in Aligarh. Only a detailed study involving purification, serology and electron microscopy can lead to a satisfactory solution of the problem. These studies are proposed to be conducted.

Chapter 6

PLAN OF WORK

Dodder transmission

Seeds of the dodder (*Cuscuta reflexa*) will be sown in 12" clay pots in sterilized mixture in the glasshouse. After germination when the plants will be about 6" long, they will be trained on tobacco plant and the tobacco plants will be inoculated after one week. After establishment of the dodder on tobacco plant, the stem of the plant will be cut. After one week a healthy tobacco plant in pot will be placed near the pot and the tips of branches of the dodder will be placed on the healthy tobacco plant when the dodder will be established on the healthy plant, the dodder will be pruned to force the movement of sap towards the healthy plant from the diseased one. The plants thus inoculated will be watched for the development of symptoms, if any, for about 6 weeks and back inoculations will be made to an appropriate test plant to confirm the presence of the virus.

Purification

All the runs will be made in a Beckman L 3-50 preparative ultracentrifuge. The first high speed run in most cases will be performed in a 30 rotor, centrifuged for 3 hr. at 78000 g. All the subsequent high speed runs will be performed in a 50 rotor at 150,000 g for two hrs. Low speed centrifugation will be done either in Remi T-24 or in Jenetzki type T-13 centrifuge.

Density gradient centrifugation

Gradient columns will be prepared by the method of Brakke (1960). Linear sucrose gradient columns will be prepared by layering 7, 7, 7 and 4 ml of 0.1M phosphate buffer pH 7.0 having 400, 300, 200 and 100 mg sucrose per ml, respectively, in a 1 x 3" tube. The sucrose solutions of different concentrations will be layered using a pipette with a broad orifice. The heaviest solution will be layered first, and solutions of decreasing concentration will be layered on the top of each other. The columns will be used after standing for 24 hr in a refrigerator. Usually 2 ml of the virus preparation will be floated on top of the column and columns will be centrifuged immediately after the virus suspension was floated on them to avoid droplet sedimentation. The columns will be centrifuged in SW 25.1 rotor in L 3-50 preparative ultracentrifuge. The acceleration upto a few hundred rpm will be done gradually. The tubes will be centrifuged for different lengths of time but later they will be centrifuged for 4 hr. After centrifugation the tubes will be examined in a dark room by projecting a narrow beam of light down the tube from the top. The virus zone will be removed from the tubes by a 20 gauge 10 cm long needle bent twice at right angles and attached to a hypodermic syringe.

Analytical ultracentrifugation

Sedimentation studies will be done in a Spinco model E analytical ultracentrifuge using Schlieren optics. All runs will

be made at a temperature of 20°C.

UV absorption spectrum

Preparations will be examined in a Beckman DU-2 model ultraviolet absorption spectrophotometer.

Isolation of RNA

The RNA will be isolated using the phenol detergent method. To a 2.5 ml solution of virus isolated from 100 gm of infected leaf tissue, will be added 0.05 ml of 6 per cent sodium dodecyl sulphate and 2.6 ml of water saturated phenol. The phenol used will be redistilled and stored at 10°C after adding distilled water. The mixture will be stirred in a glass tube on a magnetic stirrer for 10 minutes after which it will be centrifuged for 5 minutes at 300 rpm in a clinical centrifuge. The mixture will separate in two layers, the aqueous top phase will be drawn off with a pipette and 2.5 ml phosphate buffer (0.1M), pH 7.0 will be added to the lower phase. It will be stirred for 10 minutes and will be centrifuged for 5 min at 3000 rpm. The aqueous phase will be drawn off and both aqueous phases will be pooled together and stirred for 10 minutes with an equal volume of phenol followed by centrifugation. The aqueous phase will be extracted once more with one half the volume of phenol. Traces of phenol will be removed from the aqueous phase by four extractions with ether. The RNA will be precipitated by the addition of 2 ml of ice cold ethanol to the solution. The precipitate will be pelleted by centrifugation for 15 minutes at 7500 rpm. The pellet will be

suspended in 0.1M phosphate buffer pH 7.0 and centrifuged for 15 minutes at 10,000 rpm to remove any insoluble material present in the preparation.

Electron microscopy

For electron microscopy, purified virus preparation will be used. Negatively stained preparations will be prepared by employing potassium phosphotungstate (PTA). PTA stained preparations will be prepared by placing a small droplet of the virus suspension with a fine pipette on a formvar coated copper grid with a caroon backing and adding a small droplet of 2 per cent PTA solution adjusted to pH 7.0. The excess fluid will be sucked off by a piece of filter paper leaving a thin film of fluid on the grid. Then the specimens will be examined in an electron microscope.

Serology

Purified virus preparation obtained by 3 cycles of low and high speed centrifugation will be injected into rabbits. Two ml of the virus suspension will be injected intravenously. Injections will be given at one week interval. After 10 intravenous injections 3 ml of the virus suspension will be emulsified with equal amount of Freund's incomplete adjuvent and will be injected intramuscularly. During the course of injection the rabbits will be trial bled to check the titre and 2 week after the intramuscular injection rabbit will be finally bled. The antiserum will be tested and stored in small ampules in a deepfreeze.

Microprecipitin test will be performed in a petridish under mineral oil to determine the titre of the virus. Twofold serial dilutions will be made of the antiserum and the antigen. Plates will be incubated at 37°C for 2 hours. Results will also be checked by incubation at room temperature for 24 hours.

For the antigen antibody reactions Ouchterlony agar gel double diffusion test (Ouchterlony, 1948, 1958, 1962) will be used. All tests will be performed without the addition of any preservative to the agar. Crude sap and purified virus preparations will be tested. Microscope slide of 75 x 25 mm will be used. Phosphate buffer (0.1M pH 7.0) will be used for making 0.8 per cent agar. Slides will be flooded with 3 ml of agar to make a bed about 3 mm high. After the wells are charged with antiserum and antigen, slides will be incubated at room temperature in a moist chamber and readings will be taken after 24 hours. Slides will be kept under observation for several days. Proper precautions will be taken in all tests to exclude the possibility of any misreadings of the results. Different solutions of the virus and antiserum will be used and tests will be repeated several times. Physiological saline (0.85 per cent sodium chloride) in distilled water will be used for making all dilutions.

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Fig. 1. Raphanus sativus L. Left, a healthy leaf; right, leaves from an inoculated plant showing mosaic mottling and dark green raised areas.

Fig. 2. Chenopodium amaranticolor Coste & Reyn. Inoculated leaves 6-8 days after inoculation showing discrete local lesions.

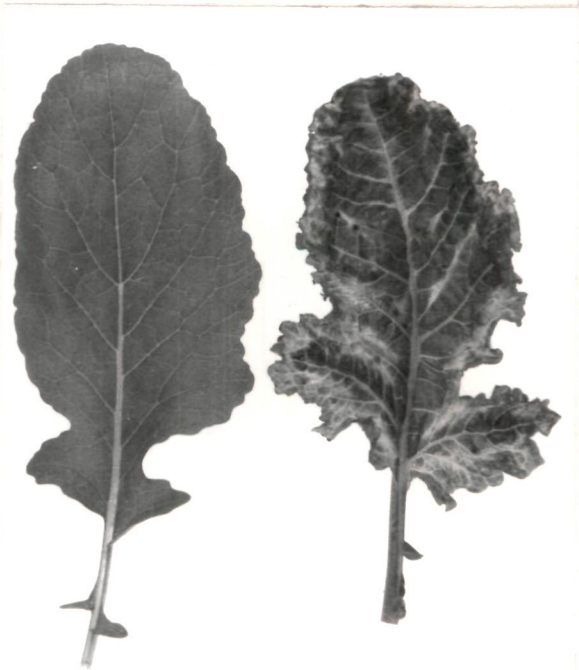

FIG.1

FIG. 2

Fig. 3. C. murale L. Inoculated leaf 6-8 days after inoculation showing local lesions.

Fig. 4. Capsicum annuum L. Left, a healthy branch; right, leaves from an inoculated plant showing mosaic and light and green areas.

FIG. 3

FIG. 4

Fig. 5. Nicotiana glutinosa L. A branch from inoculated plant showing reduction in leaf size and shoe-string formation, enations are also visible.

Fig. 6. Nicotiana tabacum var. White Burley. Left, a healthy leaf; right, leaf from inoculated plant showing mosaic and light green areas.

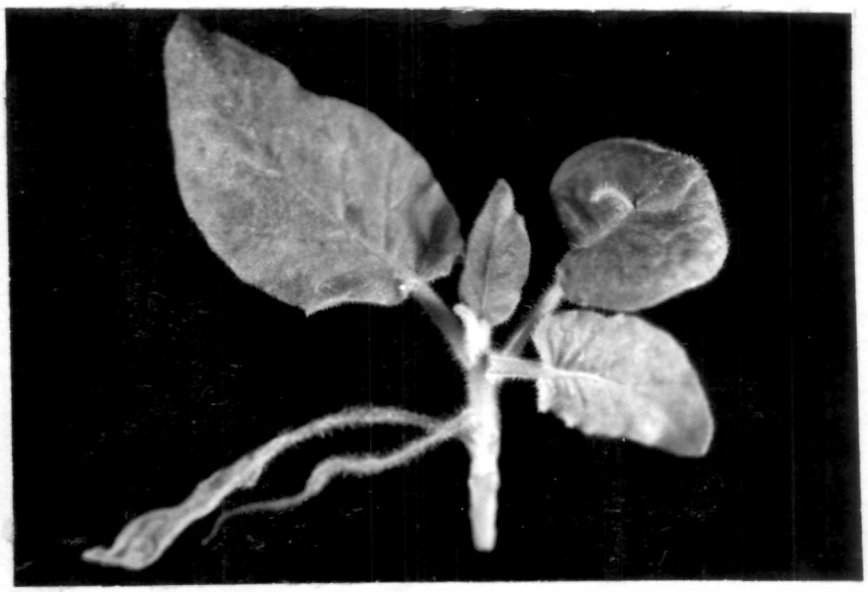

FIG. 5

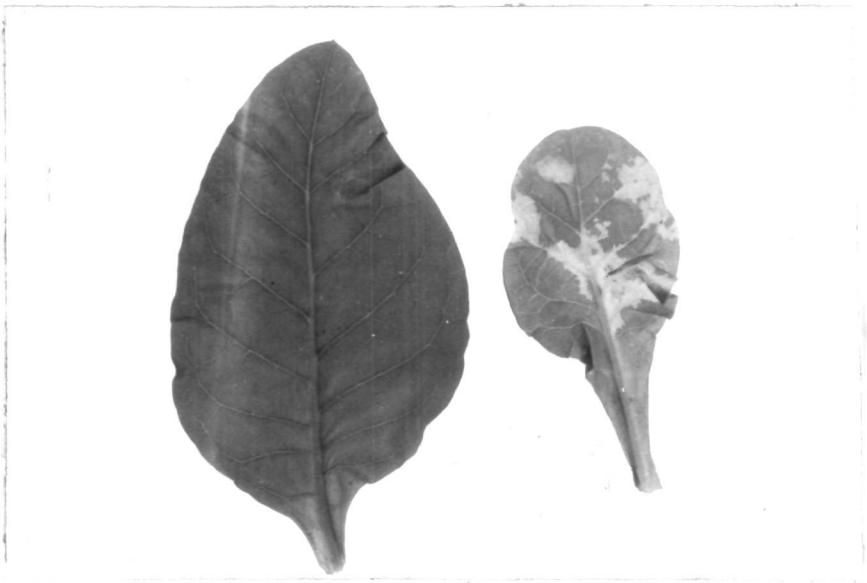

FIG. 6

Fig. 7. *N. tabacum* L. var. Harrison's Special. Left, a healthy leaf; right, a leaf from inoculated plant showing mosaic, reduction in size and deformation.

Fig. 8. *N. tabacum* cv. Turkish Xanthi. Left, a healthy leaf; right, leaf from inoculated plant showing mosaic mottling.

FIG. 7

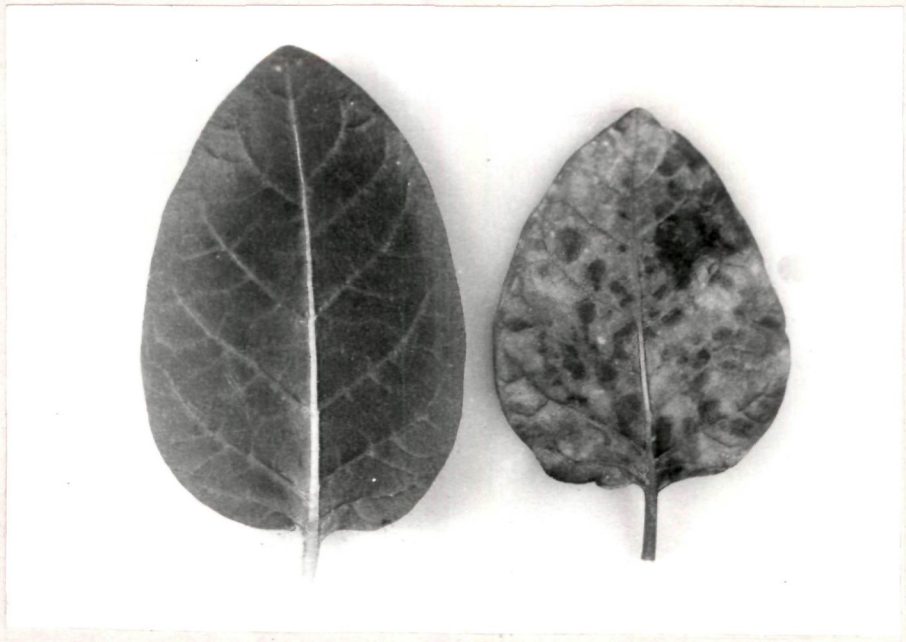

FIG.8

Fig. 9. Solanum tuberosum L. Left, a healthy leaf; right, a leaf from inoculated plant showing chlorotic lesions.

Fig. 10. Vicia faba L. Inoculated leaves showing local lesions after 6-8 days of inoculation.

FIG. 9

FIG. 10